

Investigations on the Toxic & Teratogenic Effects of GRAS Substances on the  
Developing Chick Embryo-Tail USP (Magnesium L-Alanate) No Date

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Investigations on the Toxic and  
Teratogenic Effects of GRAS  
Substances on the Developing Chick Embryo.<sup>1</sup>

*Talc USP (Magnesium Silicate)*

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<sup>1</sup>Report of investigations conducted under Contract No. 72-343 with the  
Food and Drug Administration, FDS, DDM.

## General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table 1. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, solubility of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by autoclaving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway Model 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 13 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced to 98.5° F dry bulb reading and humidity was increased to a 90° F wet bulb reading. Upon hatching (22nd day) chicks were examined for abnormalities and samples were cleared and alizarin stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, muscle, bursa, brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embedded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in duplicate and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected embryos and untreated control groups of eggs were used with each experiment. In addition, extra trials were conducted to provide embryos for examination at critical doses of the test substances in order to further evaluate teratogenic potential and obtain additional data on the nature of embryonic defects.

Table i

FDA Project Test Substances

<u>Test Substance and Identification</u>	<u>Compound No.</u>
1. Lactose, Edible Fornost Dairies, Inc. Appleton, Wisc.	000063423
2. Propyl Gallate Lot 337	000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 955102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3157 73(C)	000134032
4. Sodium Erythorbate F.C.C. Lot No. 834072 FDA 3157 73(C) Hoffmann-LaRoche, Nutley, N. J.	977052064
5. Oil Nutmeg NF, East Indian Fritzsche Dodge & Olcott, Inc. 71-28 New York, N. Y.	MX 8008455
6. Zinc Sulfate - Rayon Lot # 2132R1 Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. 007733020 Monohyd. 007446197
7. Stannous Chloride, AR 2H <sub>2</sub> O Mallinckrodt Chemical Works St. Louis, Mo.	007772998
8. Talc NF #141, Whitaker, Clark and Daniels, Inc.	010101390
9. Carob Bean Gum FDA 71-14	PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 White Chemical Organics Division New York, N. Y. FMOL D7C-30C	977051323

#### General Observation and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 48 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and arab. resin gum. Moderate toxicity was encountered with sodium ascorbate, sodium orthophosphate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table 11

Comparison of Ten Substances Tested  
for Toxicity and Teratology

Substance Tested	LC <sub>50</sub> via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	84 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micro- melia, torticollis, celosomia.
Phosphated Mono- and Di-Glycerides	>3000 mgs./kg.	Ascites, anophthalmia, brachygnathia.

## VIII. TALC USP

## Specific Protocol:

Talc is an extremely insoluble compound. After many attempts to find a suitable suspending medium, a mixture of equal parts by volume of glycerol and sterile water was used as the vehicle for administration. Five to 20 mg levels of talc were used via the air cell at 0 hr., the albumen at 96 hrs., the yolk at 0 hr. and the yolk at 96 hrs. Data using the air cell at 96 hrs. are not reported due to very high solvent toxicity.

## Results:

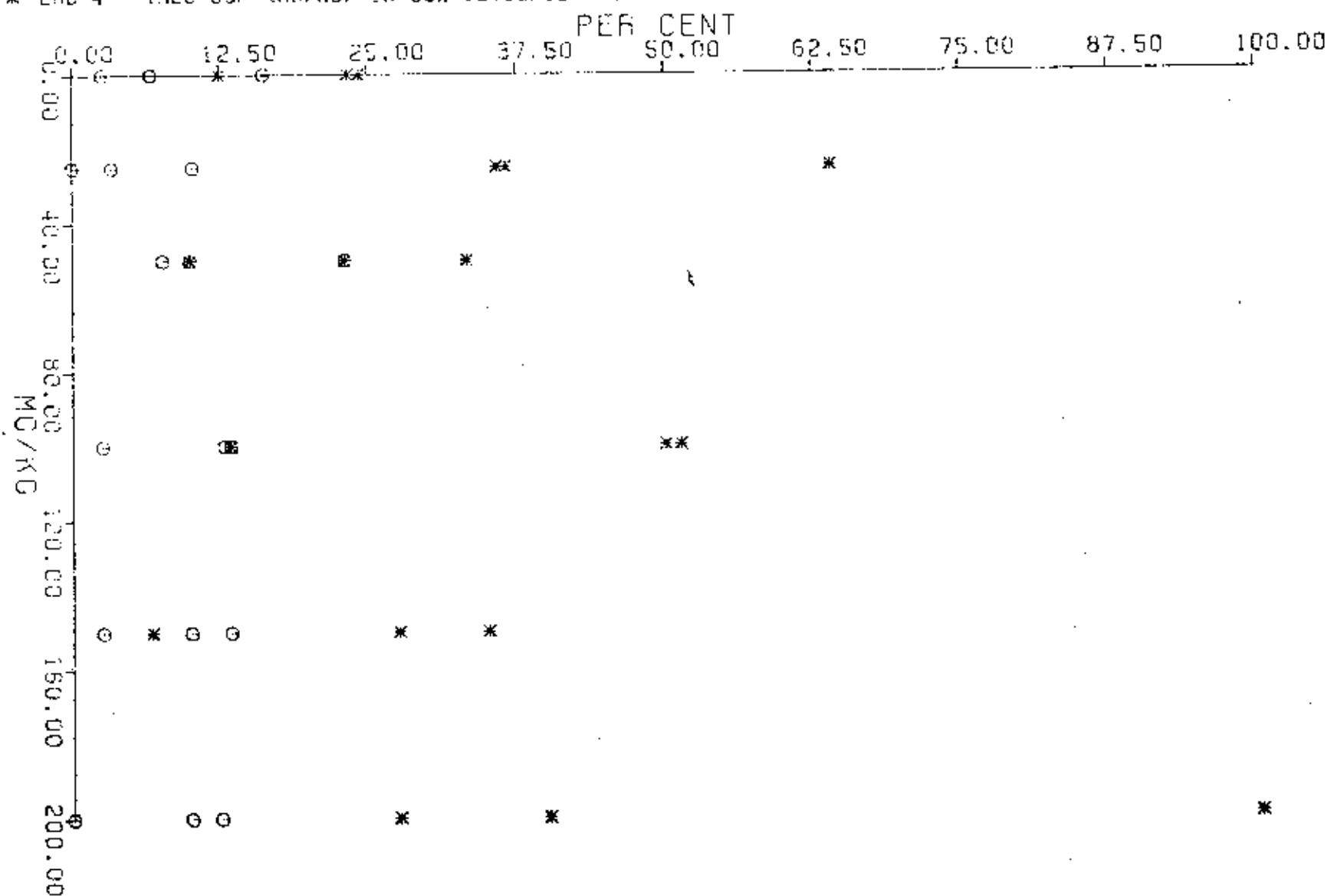
The data for the talc is presented in Tables 29-32. Percent mortality was significantly increased by the 3 highest levels of talc when given at 0 hr. via the air cell. When given in the albumen at 96 hrs. the results indicate some increase in mortality due to talc but the increase was significant only at the lowest and highest level of compound administration. High solvent control mortality was experienced at 0 hr. with yolk injection and as a partial consequence mortality was actually reduced significantly by the two highest levels of talc. When given at 96 hrs. via the yolk mortality was higher at all levels of talc injection but reached significance only at the lowest, median and highest level of administration. In all cases, there was no significant regression of dose on mortality. Percent abnormal chicks hatched was increased only by 0 hr. air cell injection at the 3 highest talc levels and not by other methods of administration. Percent H-S-V-I abnormalities was increased significantly only by air cell injection at 0 hrs. with 5.0 mgs./egg of talc and by 0 hr. yolk injection with 10.0 mgs./egg.

Unlike the data from most of the previous compounds no apparent increase in specific embryo abnormalities due to treatment with talc was noted. Embryo toxic response in the form of edema and ascites was not found in talc treated eggs to a greater extent than in the solvent controls. Some increase in celosomia may account for the overall effects observed.

## Discussion:

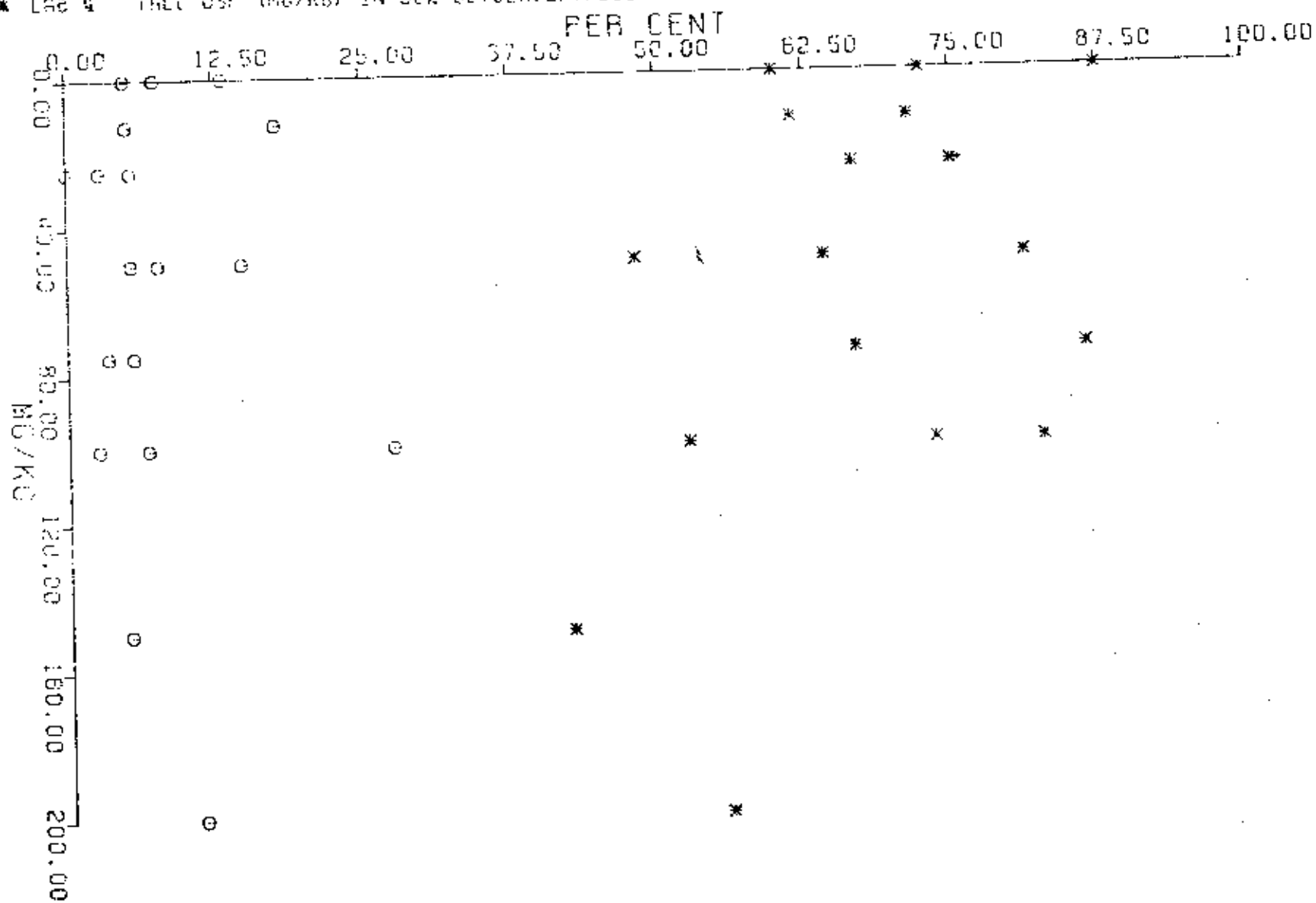
Talc failed to produce a clear cut effect on embryonic mortality or incidence of abnormalities although some significant increases in mortality and abnormalities were noted. The effect of talc would appear to be related to its physical properties rather than to its chemical properties. It's insolubility and lack of embryo toxic response suggest that it may produce some mechanical damage. If talc can be said to have an  $LD_{50}$  it would be  $>200$  mgs./kg.

O LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/Y/096 ONE OR MORE ABNORMALITIES  
 \* LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/Y/096 MORTALITY PCT

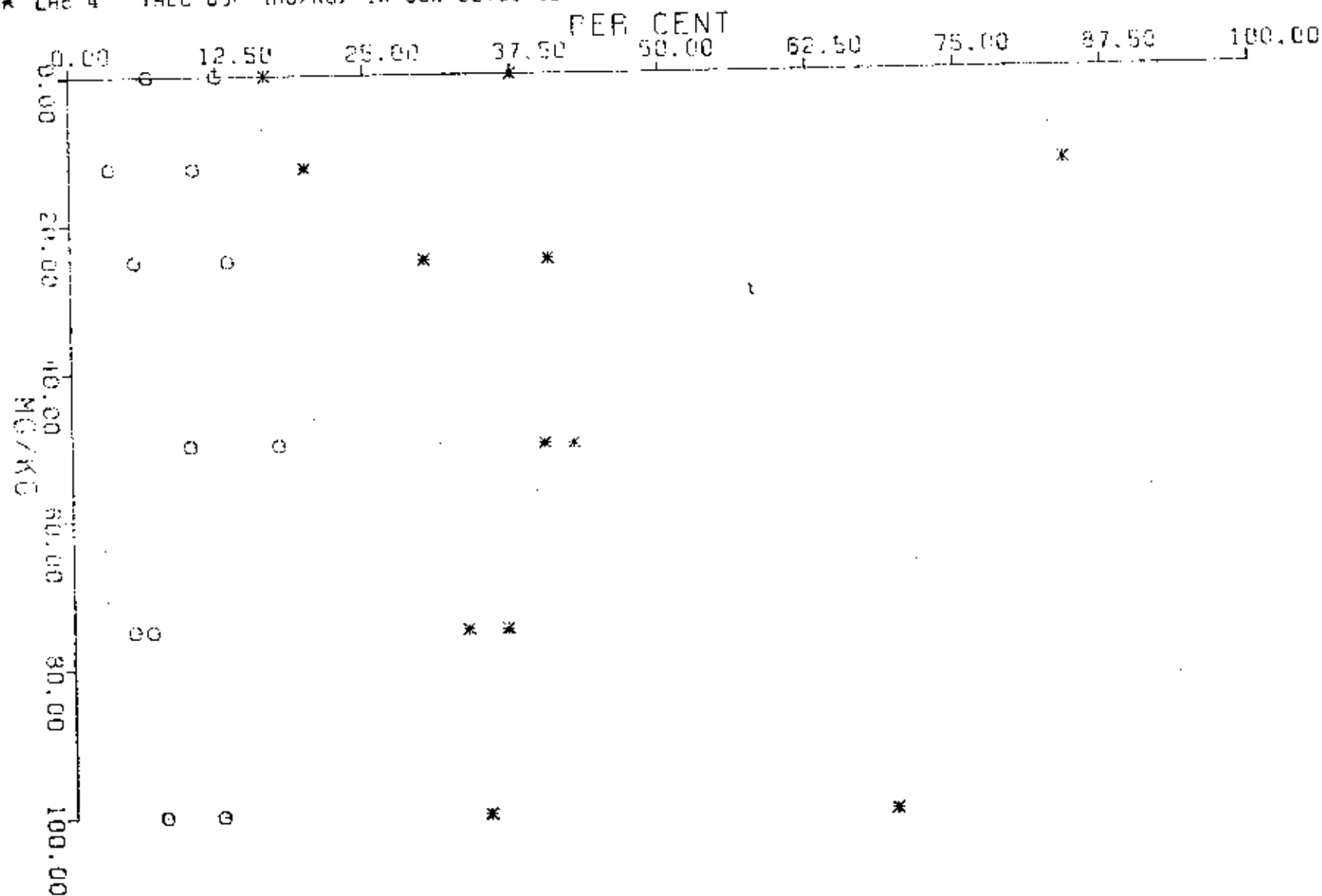


○ LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/Y/000 ONE OR MORE ABNORMALITIES

\* LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/Y/000 MORTALITY PCT



O LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/W/095 ONE OR MORE ABNORMALITIES  
 \* LAB 4 TALC USP (MG/KG) IN 50% GLYCEROL/W/095 MORTALITY PCT

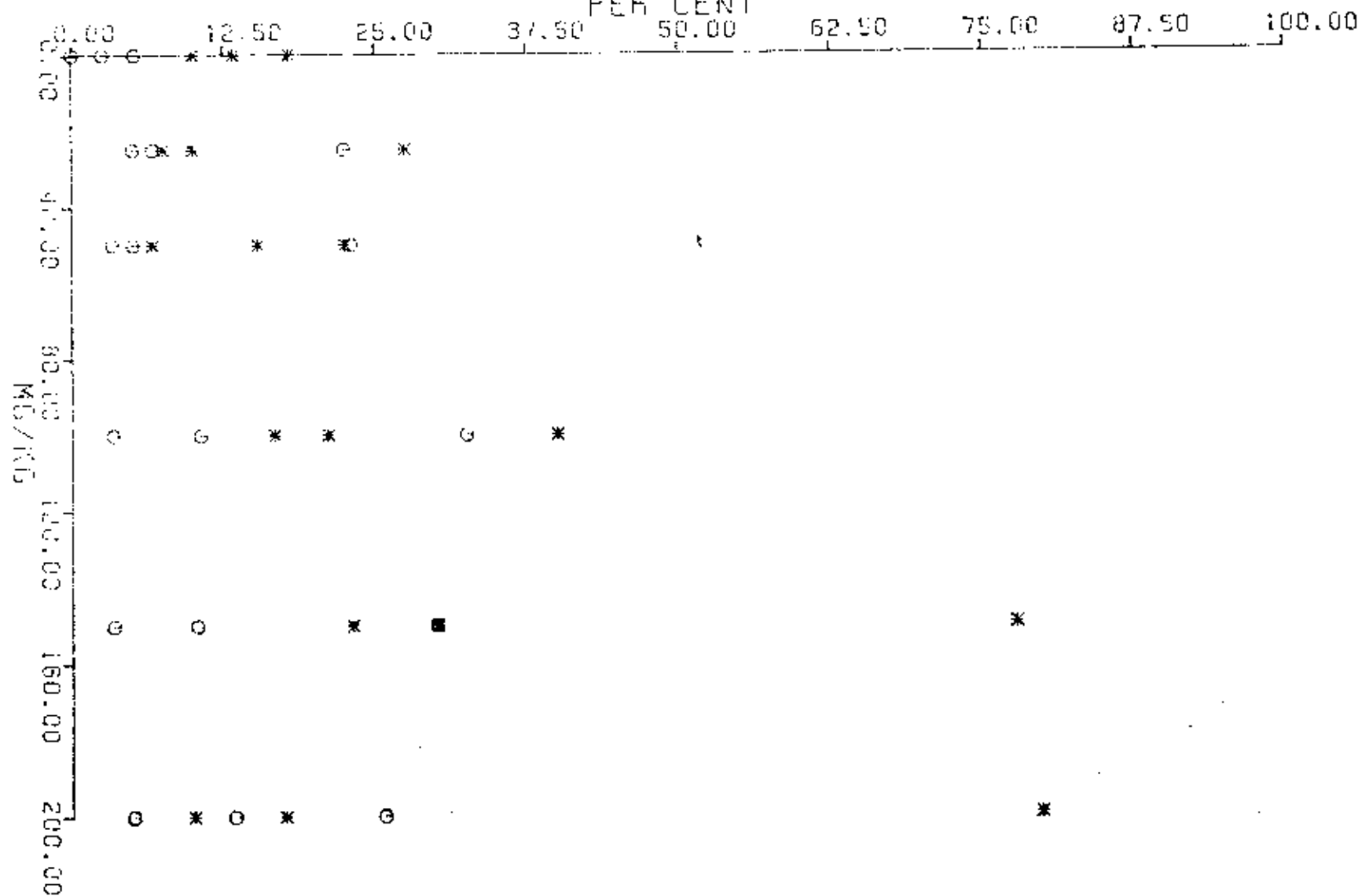


71

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\* LAG 4 TREC USP (MG/KG) IN 50% GLYCEROL/W/0.00 MORTALITY PCT

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Table 29

## DATA SUMMARY

Talc in 50% Glycerol  
via Air Cell at 0 hr.

Dose of Compound Injected (mgs./kg.)      (mgs./egg)		Number of Eggs	Percent Mortality <sup>4</sup>	Percent Abnormal Chicks <sup>5</sup> Hatched	Percent H-S-V-L Abnormalities
Control	None	434	9.90	6.45	0.92
Solvent	None	109	13.76	6.42	0
25.0	1.25	110	15.45	11.81	1.81
50.0	2.50	109	15.59	11.00	3.66
100.0	5.00	108	26.85 <sup>1a</sup>	16.66 <sup>2a</sup>	5.55 <sup>3</sup>
150.0	7.50	109	44.95 <sup>1</sup>	19.26 <sup>2</sup>	4.58
200.0	10.00	109	37.61 <sup>1</sup>	19.26 <sup>2</sup>	1.83

<sup>1</sup> Difference from control group is highly significant

<sup>1a</sup> Difference from control group is significant

<sup>2</sup> Difference from control response is highly significant

<sup>2a</sup> Difference from control response is significant

<sup>3</sup> Same as 2a

<sup>4</sup> MS - F (Cal) < F (.05)

<sup>5</sup> MS - F (Cal) < F (.05)

Table 30

## DATA SUMMARY

Falc in 50% Glycerol  
via Albumen at 96 Hrs.

Dose of Compound Injected (mgs./kg.) (mgs./egg)		Number of Eggs	Percent Mortality <sup>4</sup>	Percent Abnormal Chicks <sup>5</sup> Hatched	Percent H-S-V-L Abnormalities
Control	None	434	9.90	6.45	0.92
Solvent	None	70	28.57	14.28	2.85
12.5	0.625	68	55.88 <sup>1</sup>	7.35	2.94 <sup>3</sup>
25.0	1.25	67	35.82	8.95	1.49
50.0	2.5	70	41.42	14.28 <sup>2</sup>	1.42
75.0	3.75	69	34.78	8.69	0
100.0	5.0	66	48.48 <sup>1a</sup>	10.60	0

<sup>1</sup> Difference from control group is highly significant

<sup>1a</sup> Difference from control group is significant

<sup>2</sup> NS

<sup>3</sup> NS

<sup>4</sup> Slope is negative

<sup>5</sup> NS - F (Cal) < F (.05)

Table 31

## DATA SUMMARY

Talc in 50% Glycerol  
via Yolk at 0 Hr.

Dose of Compound Injected (mgg./kg.) (mgg./egg)		Number of Eggs	Percent Mortality <sup>4</sup>	Percent Abnormal Chicks <sup>5</sup> Hatched	Percent H-S-V-L Abnormalities
Control	None	434	9.90	6.45	0.92
Solvent	None	110	74.54	8.18	0
12.5	0.625	67	65.67	14.92	2.98
25.0	1.25	101	72.27	2.97	0.99
50.0	2.50	103	66.01	8.73	0.97
75.0	3.75	65	75.38	4.61	1.53
100.0	5.00	110	69.09	12.72	4.54
150.0	7.50	40	42.50 <sup>1</sup>	15.00 <sup>2</sup>	0
200.0	10.00	36	55.55 <sup>1a</sup>	11.11	8.33 <sup>3</sup>

<sup>1</sup> Difference from control group is highly significant

<sup>1a</sup> Difference from control group is significant

<sup>2</sup> NS

<sup>3</sup> Difference from control group response is significant

<sup>4</sup> slope is negative

<sup>5</sup> NS - F (Cal) < F (.05)

Table 32

## DATA SUMMARY

Talc in 50% Glycerol  
via Yolk at 96 hrs.

Dose of Compound Injected (mg./kg.)      (mg./egg)		Number of Eggs	Percent Mortality <sup>4</sup>	Percent Abnormal Chicks <sup>5</sup> Hatched	Percent H-S-V-L Abnormalities
Control	None	434	9.90	6.45	0.92
Solvent	None	107	19.62	11.21	0.93
25.0	1.25	108	46.29 <sup>1</sup>	4.62	0
50.0	2.5	108	23.14	13.88 <sup>2</sup>	3.7
100.0	5.0	109	40.36 <sup>1</sup>	9.17	3.66
150.0	7.5	110	24.54	3.18	0.90
200.0	10.0	105	49.52 <sup>1</sup>	8.57	4.76 <sup>3</sup>

<sup>1</sup> Difference from injected control is highly significant

<sup>2</sup> NS

<sup>3</sup> NS

<sup>4</sup> slope is negative

<sup>5</sup> NS